Faculty of Resource Science and Technology

PRODUCTION OF HIGH PROTEIN FUNGAL BIOMASS (HPFB) IN SHRIMP POND WATER: EFFECTS OF AERATIONS

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DECLARATION

I hereby declare that this Final Year Project Report entitle "**Production of High Protein Fungal Biomass in Shrimp Pond Water: Effects of Aerations**" is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been submitted in support of an application for another degree or qualification to this or any other university or institute of higher learning.

(Christine Rika anak Renggu)

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LIST OF ABBREVIATION

R. oligosporus	*Rhizopus oligosporus*
mg	milligram
ml	millilitre
YMB	Yeast Malt Broth
L	Liter
vvm	volume per medium
g	grams
hrs	hours
rpm	rotation per minute
Zn	Zinc
NaCl	Sodium Chloride
nm	nanometer
°C	Degree Celsius
HPFB	High Protein Fungal Biomass
SmF	Submerged fermentation
%	Percentage

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Production of High Protein Fungal Biomass in Shrimp Pond Water: Effects of Aerations

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ABSTRACT

As the agricultural industries are developing over the years, the discharged of untreated waste materials are also on the rise. One of the examples is the discharge of unwanted wastes from shrimp aquaculture. High levels of toxic materials such as nitrogen and phosphorus lead to pollutions towards the environment. Due to this matter, shrimp pond water was treated by using *Rhizopus oligosporus* to treat the organic matters present in the substrates by using submerged fermentation method. Based on this research, different rate of aerations were used to test the growth of *R. oligosporus*. Different parameters included were 0.00 vvm, 0.50 vvm, 0.75 vvm and 1.00 vvm rate of aerations. 1.00 vvm aerations produced the highest dry biomass of 4.60 g/L followed by 0.75 vvm, 0.50 vvm and 0.00 vvm. The highest rate of aerations had the highest production of biomass as the microorganism was able to grow with sufficient oxygen provided. The analysis that were done in this research were phenol-sulphuric analysis, nitrate and nitrite analysis. For the phenol-sulphuric analysis, 1.00 vvm aeration had the highest amount of reduction with 93.6% compared to the other three parameters. Other than that, both nitrate and nitrite analysis also showed that 1.00 vvm had the lowest concentration of 0.52 mg/L and 0.00 mg/L respectively. This proved that *R. oligosporus* was able to treat the shrimp pond water after days being introduced to the substrate. Moreover, the high protein fungal biomass produced could be used as animal feeds which is highly in demand at the global food industry other than reducing rate of environmental pollutions.

Keywords: *Rhizopus oligosporus*, shrimp pond water, aerations, submerged fermentation, high protein fungal biomass (HPFB)

ABSTRAK

Sektor perindustrian pertanian semakin membangun dari tahun ke tahun yang menyebabkan sisa buangan juga meningkat sejak kebelakangan ini. Salah satu contoh sisa buangan ialah dari air kolam udang. Tahap bahan toksik yang tinggi seperti nitrogen dan fosforus menyebabkan berlakunya pencemaran terhadap alam sekitar. Oleh sebab itu, air kolam udang telah dirawat dengan penggunaan Rhizopus oligosporus untuk membantu merawat bahan organik yang terdapat dalam substrat tersebut menggunakan cara fermentasi substrat cecair. Berdasarkan kajian ini, jumlah pengudaraan yang berbeza telah digunakan untuk mengkaji pertumbuhan R. oligosporus. Parameter yang berbeza termasuklah 0.00 vvm, 0.50 vvm, 0.75 vvm dan 1.00 vvm tahap pengudaraan.1.00 vvm pengudaraan telah menghasilkan biomas yang tertinggi iaitu 4.60 g/L yang diikuti oleh 0.75 vvm, 0.50 vvm dan 0.00 vvm. Pengudaraan yang tertinggi mempunyai produksi biomas yang tertinggi kerana mikroorganisma dapat membantu pertumbuhan dengan tahap oksigen yang mencukupi. Antara analisis yang digunakan dalam kajian ini ialah fenol-sulfurik, nitrat dan nitrit. Bagi analisis fenol-sulfurik, penggudaraan 1.00 vvm telah mencatatkan tahap reduksi yang tinngi sebanyak 93.6% berbanding dengan tiga parameter yang lain. Selain itu, kedua-dua analisis nitrat dan nitrit juga menunjukan bahawa 1.00 vvm mempunyai tahap konsentrasi yang rendah iaitu 0.52 mg/L dan 0.000 mg/L. Hal ini membuktikan bahawa R. oligosporus berpotensi untuk merawat air kolam udang selepas beberapa hari bertindakbalas dengan substrat. Selain itu, biomas kulat berprotein tinggi (HPFB) yang dihasilkan dapat digunakan sebagai bahan mentah makanan haiwan ekoran daripada permintaan yang tinggi dalam industri pemakanan di peringkat global selain mengurangkan pencemaraan alam sekitar.

Kata kunci: Rhizopus oligosporus, air kolam udang, pengudaraan, fermentasi substrat cecari, biomas kulat berprotein tinggi (HPFB).

CHAPTER 1

INTRODUCTION

1.1 Introduction

Shrimp aquaculture is one of Malaysian's important agricultural as the industry is highly in demand both national and international market (Ismail and Abdullah, 2013). Many coastal areas are now developed into aquaculture ponds due to the increase of protein needs and also act as an alternative for reducing landing of captured shrimp (Nyanti *et al.*, 2011). According to Ghee-Thean *et al.* (2016), most consumers preferred white meat such as shrimp and fish products compared to red meat which is ruminant-based products. The Ministry of Agriculture Malaysia (2003) stated that the Malaysian government has taken an initiative to promote the brackish water shrimp culture with a target of RM 4.3 billion of shrimp production in year 2010 (Islam *et al.*, 2014).

Based on Table 1, it shows the statistics of white shrimp production from year 2002 until year 2012. The number of white shrimp produced increased gradually from year 2002 to year 2010. Meanwhile, it started to show a decreased from year 2011 onwards.

Table 1 Annual Malaysian white shrimp production. (**Source** Annual Fisheries Statistics, 2002-2012.)

Year	White shrimp (metric tons)	Pond size (ha)	Productivity (metric tons/ha/year)	Productivity (metric tons/ha/year)
2002	844.46	246.89	3.420	1.710
2003	803.59	192.21	4.181	2.091
2004	5117.49	1201.59	4.259	2.130
2005	11497.80	2391.37	4.808	2.404
2006	18600.59	2733.71	6.804	3.402
2007	23737.40	4808.63	4.936	2.468
2008	37544.31	4691.23	8.003	4.002
2009	52926.42	4823.63	10.972	5.486
2010	69084.10	5132.85	13.459	6.730
2011	60322.01	5828.71	10.349	5.175
2012	48991.81	5349.17	9.159	4.580

However, the shrimp aquaculture also has its own consequences as increase in market demands brought up negative impacts especially towards the environments (Nyanti et al., 2011). Paez-Osuna *et al.* (2003) and Primavera (2006) both stated that the negative impacts include the damage on coastal ecosystem followed by reduce amount of fisheries products and increase in surrounding pollutions caused by discharged of shrimp ponds.

Shrimp aquaculture creates many negative impacts due to illegal pond sediment disposal, pond drainage during the harvesting and also large loading of oxygen-consuming organic matters (Dierberg *et al.*, 1996). The development of shrimp agriculture also lead to shortage of clean water supply in which becomes a crucial matter as the surrounding becomes acidic due to release of heavy metals from the sediments in shrimp pond water (Kautsky *et al.*, 2000).

On the contrary, few studies had been done to treat any unwanted disposal of agricultural wastes. One of the ways is by using fungi treatment. Lennartson *et al.* (2014), mentioned that the *Rhizopus* genus has also been investigated for treatment of industrial wastewater. The wastewaters such as shrimp pond water could be used as organic sources and aid in the production of animal feed. Anupama and Ravindra (2000), stated that the bioconvension of agricultural and industrial wastes to a rich protein food has been giving a positive results in producing a cheaper product and yet reducing wastes pollutions.

Since 500 years ago, the *Rhizopus* genus has been used in full-scale industrial applications especially in the warm and moist Southern Asia compared to colder Northern Europe (Lennartson *et al.*, 2014). One long-time species in use is the fungi *Rhizopus oligosporus* in tempeh, a dish from fermented soybeans (Jennessen *et al.*, 2008).

According to Mitra *et al.* (2012) and Rasmussen *et al.* (2014), *R. oligosporus* can be used for the removal of organic compounds from thin stillage. Fungal cultivation also assist in the recycling of water and to reduce water evaporation that is low in energy cost. The fungi that have been growing in thin stillage can then be harvested, dried and used as animal feed (Nitayavardhana *et al.*, 2013).

Mitra *et al.* (2012) and Ramussen *et al.* (2014) also stated that thin stillage acts in providing nutrients such as organic compounds and micronutrients that makes it an ideal feed stock for fungal cultivation such as lactic acid. Animal feed is important in the global food industry in order to increase products which originated from animals throughout the world.

Moreover, Jaganmohan *et al.* (2013) stated that research on high protein fungal biomass (HPFB) production in animal feed has been done due to shortage of essential amino acids for synthesizing of protein in animals in the early days. Increasing of protein synthesis can help in providing nutrients and enhance feeding efficiency for the animal. Submerged fermentation is one of the methods that can help in production of HPFB. It can aid in the agricultural industry in terms of animal feed. At the end of the submerged fermentation process, it also can help in reducing the production cost (Zhang *et al.*, 2008).

In this research, *Rhizopus oligosporus* was used and grown in shrimp pond water by using submerged fermentation method to treat and reduce organic matters present in shrimp pond at the same time producing HPFB.

The specific objectives of this research are:

1. To determine the effects of different rate of aeration on *R. oligosporus* growth.
2. To investigate the effectiveness of shrimp pond water treatment using *R. oligosporus*.
3. To identify the optimal aeration rate for growth of *R. oligosporus* in shrimp pond water.

1.2 Problem Statement

There are many organic materials that are present in shrimp pond water that leads to contamination of water. Due to this matter, this research is conducted in order to remove this unwanted materials from shrimp pond water. *R. oligosporus* was used to reduce contaminating substances and produced HPFB to be used as animal feed. We investigated the growth rate of *R. oligosporus* for the production high protein fungal biomass (HPFB) in shrimp pond water that acts as medium to provide nourishment for the *R. oligosporus* at different levels of aeration.

CHAPTER 2

LITERATURE REVIEW

2.1 *Rhizopus oligosporus*

Rhizopus oligosporus belongs to *Rhizopus microsporus* group that associated with food fermentation, pathogenesis or unwanted metabolite production like rhizoning and rhizotoxins (Jennessen *et al.*, 2008). Jennessen *et al.* (2008) stated that *R. oligosporus* has a higher defect in spore formation process with a high proportion of 10-31% larger and irregular spores compared to the other natural *Rhizopus* taxa. Their optimum growth are at 42 ˚C and pH of 4 (Medwid *et al.*, 1984). *R. oligosporus* is one of the fungal species that have the ability to produce several of proteases enzymes compared to bacteria. This cause the *R. oligosporus* been used widely in the fermentation of vegetal substrates (Kovac *et al.*, 1996). For example, it is traditionally used in producing tempeh which is a fermented food based on soybeans.

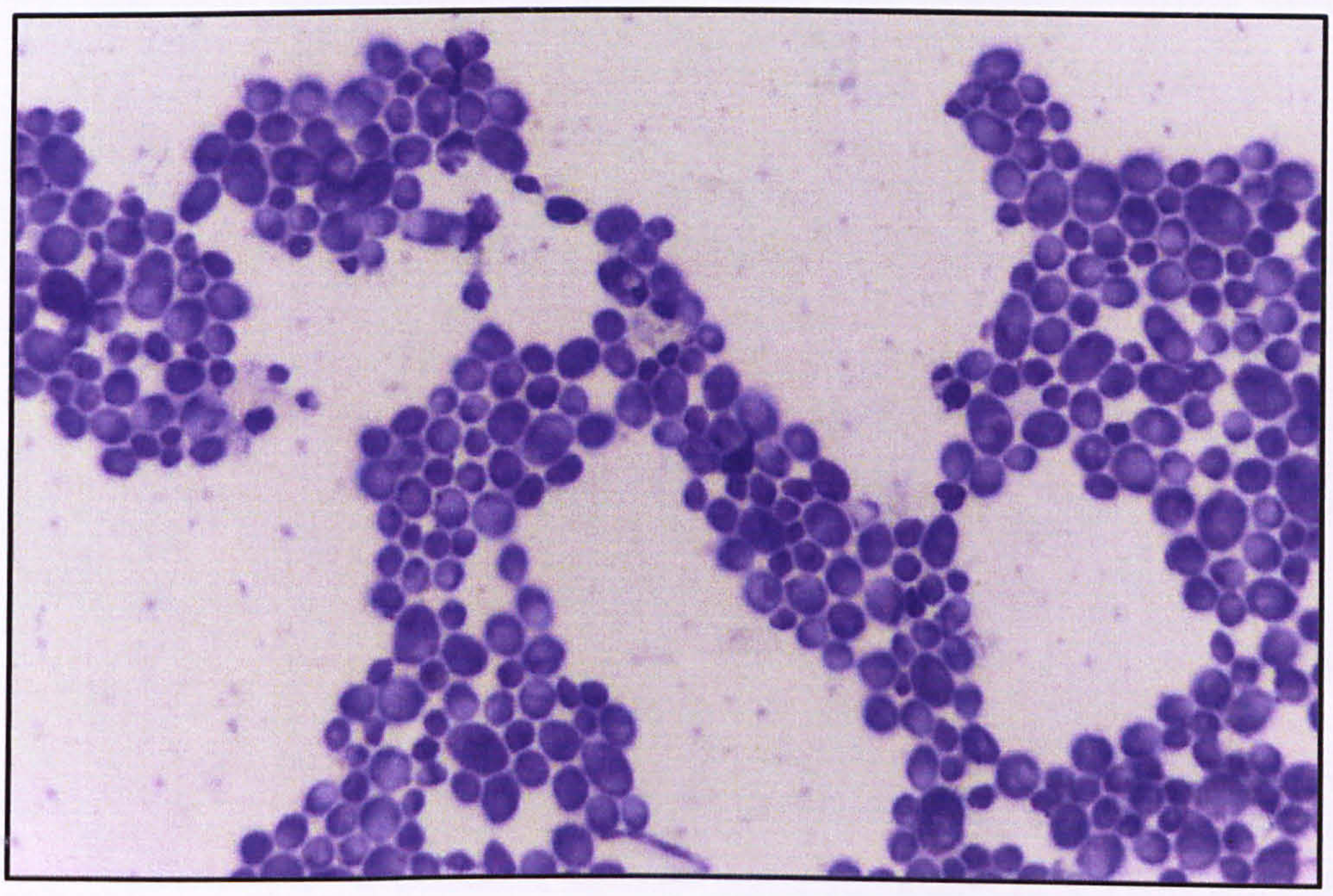

Figure 1 *Rhizopus oligosporus* viewed under light microscope. (Magnification: 1000X)

According to Hesseltine (1965), *R. oligosporus* is the main principle mold responsible species for fermentation of soybeans. Handoyo *et al.* (2006) stated that *R. oligosporus* in cultured soybeans are hydrolyse protein to amino acids and peptides by secreting several proteolytic enzymes. Meanwhile, the oligosaccharides of *Rhizopus* will be hydrolysed to monosaccharides. As for the phytic acid, it will be degraded into inorganic phosphate in the process of cultured soybeans. The synthesis of the enzymes hydrolysed contribute to the development of desirable texture, flavour and aroma of the product (Kovac *et al.*, 1996). Other than being used to ferment other substrates, it can also treat waste material (Jennessen *et al.*, 2008).

2.2 Shrimp pond water

Globally, shrimp agriculture has been developing steadily over the last few decades in response to the increasing of the world market demand. The production system evolved from extensive toward intensive with increasing inputs of high quality feed and water supply (Lin *et al.*, 1995).

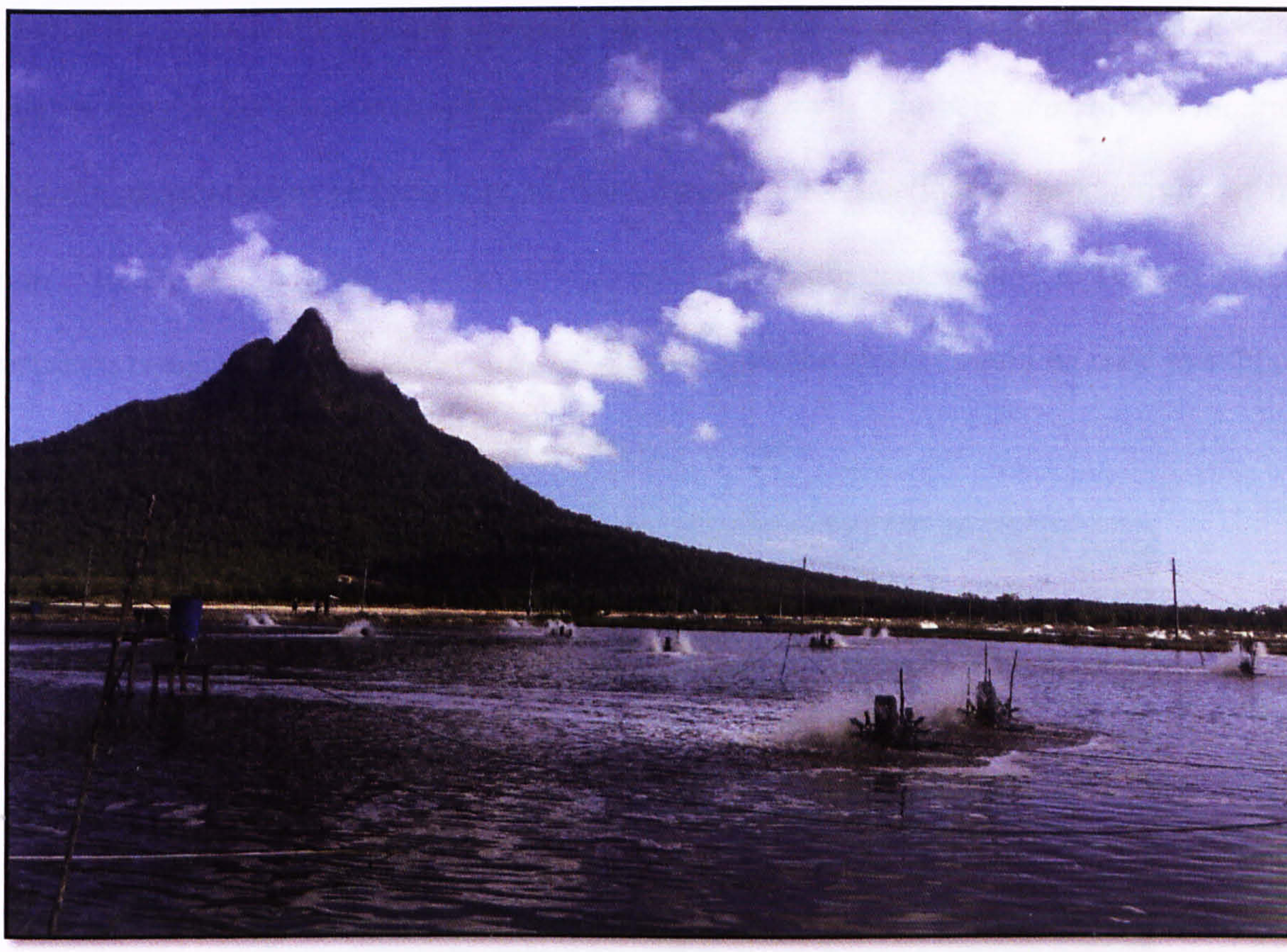

Figure 2 Shrimp pond located at Kuala Santubong, Kuching.

Nyanti *et al.* (2011), stated that the shrimp agricultural is one of the industries that are developing to the creation of Aquaculture Industrial Zone in Malaysia. Nevertheless, the increase in waste loads of uneaten feed and metabolic wastes from culture ponds, is causing the surface and subsurface salinization of freshwater and the loading of solids which also includes any oxygen-consuming organic matter (Dierberg *et al.*, 1996).

Dierberg *et al.* (1996) also stated that between 1979 until 1993, about 16 to 32 % of total mangrove area were destroyed due to shrimp agriculture. Based on Thakur *et al.* (2003), in a traditional intensive shrimp culture, the deteriorated pond water is frequently exchange with new water supply in order to maintain a desirable water quality of shrimp growth.

However, nitrogen wastes like ammonia and nitrite are produced in the system in which exceed the assimilating capacity of receiving waters. This leads to the deterioration of water quality that caused toxic environment for the shrimp growth. Dierberg *et al.* (1996) reported that the abandonment of shrimp pond water caused the reductions of productivity of the pond year by year. Nonetheless, in the study done by Thakur *et al.* (2003), in a closed system nutrient, nitrogen and phosphorus accumulated within the system could be used to support the growth of natural food organisms for shrimp growth.

Figure 3 Shrimp pond farm.

2.3 Submerged Fermentation (SmF)

Submerged fermentation (SmF) is one of a process which is used in maximizing the productivity of microorganisms such as bacteria and fungi (Subramaniyam *et al.*, 2012). In submerged fermentation free-flowing liquid substrates is usually been utilized. Due to the rapid utilization of nutrients, it also requires rapid supplementation (Mienda *et al.*, 2011). Vidyalakshmi *et al.* (2009), mentioned that a submerged culturing is an advantage because it helps in easing of sterilization and in controlling the process system. According to Chisti *et al.* (1999), submerged fermentation can be divided into oxygen requiring aerobic processes and also in anaerobic processes which in absence of oxygen. He also stated that in submerged fermentation systems, it can be carried out either as batch-wise, fed-batch operations or continuous cultures.

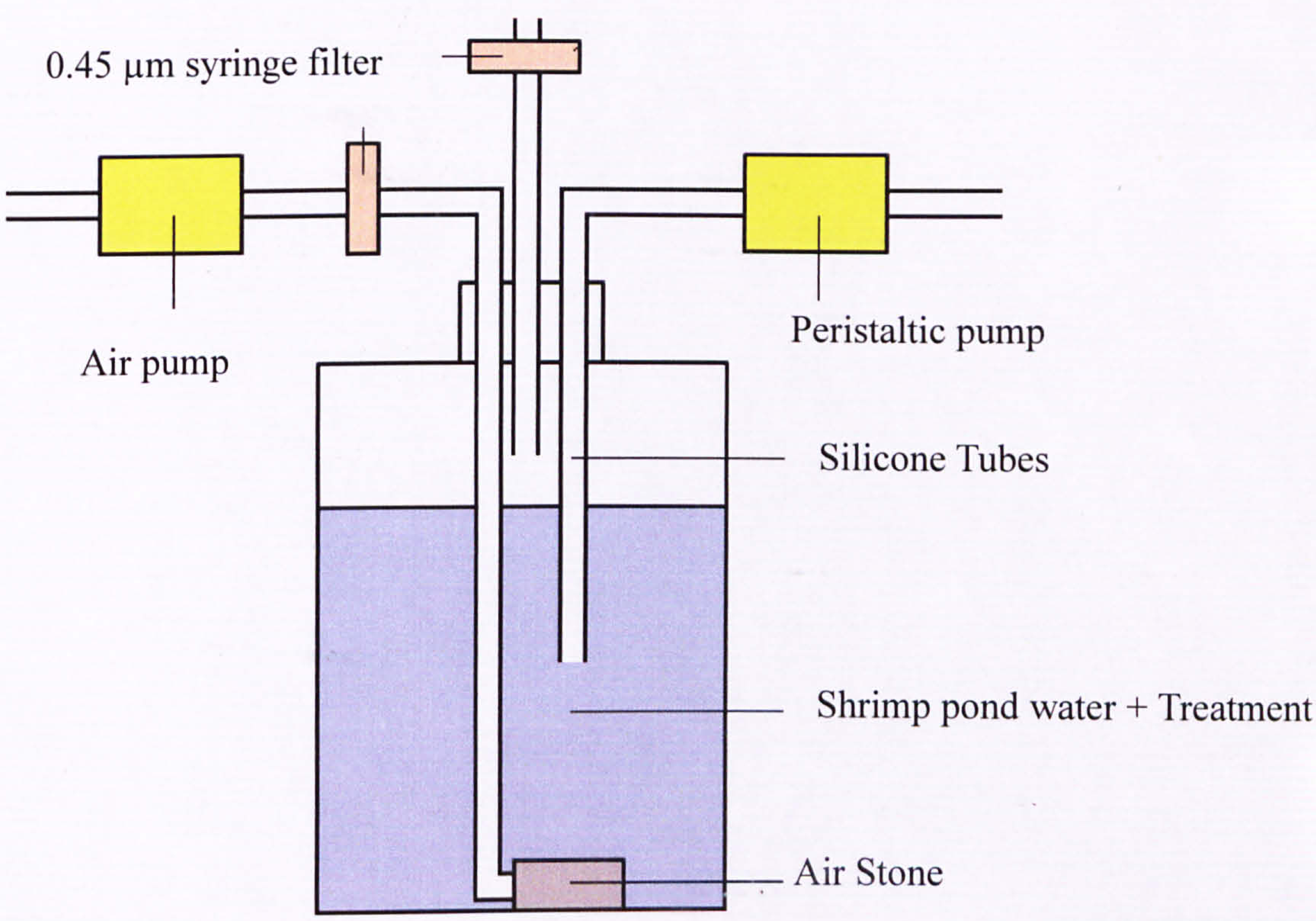

Figure 4 Set-up of submerged fermentation.

For batch processing, a batch is inoculated in a fermenter with microbes for certain amount of time for product to be harvested. As for fed-batch fermentation, culture medium will be added periodically to the inoculated fermentation batch. Product is then harvested after batch period (Chisti *et al.*, 1999).

Meanwhile, for continuous fermentation, sterile medium is fed continuously into the fermenter and product will then withdraw continuously so that the fermentation volume remain unchanged (Chisti *et al.*, 1999). It is also said that solid-state fermentation offers some advantages over submerged fermentation. Subramaniyam *et al.* (2012), mentioned that SmF is usually used for the extraction of secondary metabolites that required in liquid form.

2.4 High Protein Fungal Biomass (HPFB)

Reaction between carbon dioxide (CO_2) in the water, air and sunlight via photosynthesis in plant material produce carbohydrates which is the building blocks of biomass (McKendry, 2002) High protein fungal biomass are substances that contain a high concentration of protein with essential amino acids for both human and animal (Moore *et al.,* 2001). According to Jennessen *et al.* (2008), *R. oligosporus* has been used to produce single cell protein due to its ability to utilize carbon sources and produce a high protein yield. Other than that, it also aid in the production of animal feedstock. As protein is one of the most essential components needed for the growth and development of living things, the single cell protein (SCP) provides an alternative yet convenience way for consumption (Suman *et al.*,2015).

Figure 5 Dry biomass obtained after accumulation process.